**Reviewer’s Comments**

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***In vitro*assessment of antimicrobial and antioxidant activities of Yemeni *Fagoniaschweinfurthii*Hadidiextracts**

**Abstract:**

**Background:**Antioxidant and antimicrobialproperties of plant extracts are attributed to bioactive components derived from medicinal plants. This study investigated the antimicrobial and antioxidant properties of the aerial portions of *Fagoniaschweinfurthii*Hadidi extracts.

**Method:**Several solvents, including n-hexane, ethyl acetate, and methanol, were used sequentially to extract secondary metabolites from *F. schweinfurthii* aerial parts. The well diffusion method was used to assess antimicrobial activity, while antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method.

**Results:**The findings exposed that the studied fungal strains (*Candida albicans* and *Trichophyton rubrum*) were resistant to various plant extracts. *F. schweinfurthii* methanol extract demonstrated the most potent inhibitory effects on all Gram-positive and Gram-negative tested bacteria. In addition, the most sensitive bacterium was *Proteus vulgaris*, with an inhibitory zone measuring between 2.5 and 5 mm.

Due to the physical and chemical properties of the solvents, different extracts of *F. schweinfurthii*aerial parts exhibited diverse antioxidant capabilities in the antioxidant activity experiment. At various concentrations ranging from 62.5 to 500 g.mL-1, methanol extract demonstrated the greatest DPPH radical scavenging efficacy.

**Conclusion:**The results of this study showed that the aerial parts of *F. schweinfurthii*could be considered a potential source of natural antioxidants and a valuable source against bacteria that cause ear infections.

**Keywords**

**INTRODUCTION:**

During their physiological processes, plants produce a variety of secondary metabolites, including alkaloids, polyphenols, flavonoids, terpenoids, and carotenes, among others. In addition to serving as preventative and therapeutic agents against many diseases, these secondary metabolites also assist boost immunity1.These secondary metabolites are a potent source of anticancer 2, antioxidant 3, antiviral 4, anti-inflammatory 5, and antimicrobial agents6.

The prevalence of life-threatening infections produced by microbial pathogens has increased globally, and among developed regions, it is now a significant cause of death in immunosuppressed patients7.

In order to minimize the spread of infectious diseases worldwide, antimicrobial drugs are crucial8. Although fewer, or even occasionally, ineffective antimicrobial treatments are available for the infection caused by pathogenic bacteria, the development and spread of multidrug-resistant (MDR) strain have become a significant public health threat9,10. Folk medicine offers a valuable and underdeveloped resource for researching and developing potential new treatments for microbial infections to reduce the evolution of drug resistance and adverse medication effects. Additionally, because these may be more readily available, more economical, and more accessible, the use of medicinal plants opened the potential for the developing world11.

Free radicals are created spontaneously in biological systems as endogenous reactive oxygen/nitrogen species (ROS/RNS) as a result of normal metabolic events 12. Furthermore, severaloutside factors, including pollution, UV rays, alcohol, smoking, and chemicals, increase oxidative stress, which leads to an excess of free radicals in the human body 13. Naturally, the endogenous antioxidant defense mechanisms (SOD, catalase, and peroxidase) regulate their synthesis and lessen the excess of these substances. Various exogenous antioxidants, such as vitamins E, C, and -carotene, scavenge free radicals. However, when produced in excess, these antioxidants lose their effectiveness. Free radicals can negatively impact lipids and proteins, leading to peroxidation, which can result in unfavorable alterations to cells and gene mutation 14, premature aging, tissue damage, and inflammation 15. These free radicals ultimately cause diabetes mellitus, Alzheimer's disease, cancer, and other neurological disorders 16,17,18. Because of their capacity to stop or reduce oxidative damage, many synthetic dietary antioxidants have gained recognition. The most widely used, such as tert-butyl hydroquinone (TBHQ), butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), and tocopherol, have been banned in the food industry because they are thought to cause liver damage and cancer19. Therefore, scientists have become more interested in discovering inexpensive, safe, and natural alternatives to antioxidants. It was difficult to build the sustainability idea to screen natural, abundant, low-value raw products. Vegetables, fruits, and biomass from forests all provide significant sources of bioactive compounds, notably natural antioxidants 13,20.

*Fagoniaschweinfurthii*Hadidi (family Zyophyllaceae) and its closely related species are found throughout the deserts and dry regions of Southwest U.S.A., Chile, and tropical Africa21.People in the desert have traditionally used the plant to cure skin eruptions, ulcers, skin ailments, antipyretic, pain relief, ear infections, venereal disorders, and other conditions22. Both internal and topical preparations have traditionally been used to treat hemorrhoids, inflammation, ulcers, leprosy, open wounds, and fever. When the entire plant of *F.schweinfurthii* is boiled in water, the bath is useful for allergies and other skin disorders, and the decoction is taken orally as a blood purifier23.

The anticancer, antibacterial, antiviral, analgesic, anti-inflammatory, antipyretic, cooling, antioxidant, and thrombolytic effects of *Fagonia* species have been demonstrated 24.Phytochemical investigation suggests that *F.schweinfurthii* extracts contain alkaloids, cardiac glycosides, flavonoids, carbohydrates, tannins, saponins, steroids, and amino acids 25.Recent research26 investigated the DPPH radical scavenging activity of *F.schweinfurthii*Hadidi aerial parts ethanol extracts, where results showed antioxidant activity with an IC50 of 200.277.34 g/ml.Mothana*et al*27. reported that methanol and aqueous extractsof Yemeni *Fagonia indica*, displayed a significant concentration-dependent DPPH scavenging activity ranging from 17.6%to 85%. On the other hand, the aqueous extract demonstrated a mild concentration-dependent DPPH scavenging activity ranging from 3% to 22.7%.Al Ghanem31tested extracts of *F. mollis*in petroleum ether, methylene chloride, ethyl acetate, and methyl alcohol for antibacterial activity against pathogenic bacterial strains *(Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus)* and *fungal strains (Candida albicans, Mucor spp., Aspergillus fumigatus,* and*A.Aspergillusniger)*. However, all microorganisms were suppressed by acetone, except *Escherichia coli* and *Staphylococcus aureus.* The plant's methyl alcohol extract displayed broad-spectrum antibacterial action on studied microbes. Conversely, *Aspergillus fumigatus* and *Aspergillus niger* were unaffected by Petroleum ether extract, which inhibited the growth of *Candida albicans* and *Mucor spp.* Whilethe methylene chloride, ethyl acetate, and methyl alcohol showed no antifungal action. On the other hand, *A. fumigatus* experienced growth inhibition from acetone extract, whereas the other bacteria were unaffected.Ur Rehman et al 32 found that the methanolic fraction of *Fagoniacretica* aerial parts had the highest significant antibacterial and antifungal activities (46-57 % & 39-60 %) against tested bacterial and fungal strains.

Despite claims that*F.schweinfurthii* has antibacterial and antioxidant properties. The antioxidant and antibacterial properties of methanol, ethyl acetate, and n-hexane extracts of *F. schweinfurthii* are still unknown.

As a result, this study aims to investigate *F. schweinfurthii's*potential as an antioxidant and antibacterial agent for the treatment of ear infections.

**Materials and Methods:**

**Plant Material:**

*F. schweinfurthii* aerial parts were obtained in September 2019 from resident areas in Sana'a, Yemen. Dr. Hassan Ibrahim recognized the plant in the Biology Department, Sciences College, Sana'a University*.*

**Test Organisms:**

Two-gram positive bacterial species (Staphylococcus aureus and Staphylococcusepidermidis), gram-negative bacterial species (Escherichia coli, Proteus vulgaris), as well as two fungi, including one filamentous fungus belonging to dermatophytes (Trichophytonrubrum) and one yeast species (Candida albicans), obtained from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt, were used in this study.

**Preparation of plant extract:**

One kilogram of fresh aerial parts was cleaned thoroughly with tap water, dried at room temperature, and ground into a fine powder with an electric blender. Before subsequent applications, the powder was kept in a cool place and away from light.About 752g of powdered aerial components were soaked in 3.76 L of n-hexane, ethyl acetate, and methanol, respectively. The samples were submerged at room temperature for three to seven days with constant stirring. This procedure was repeated three times to increase the extraction efficiency; following filtration, the extracts were concentrated at a temperature of 40 °C in a rotary evaporator and dried in an oven at 37 °C.For further examination, the dried extracts were weighed and kept at 4°C.

***Invitro* antimicrobial activity assay:**

1. **Agar Well Diffusion Assay**

The agar well diffusion method was used to investigate antimicrobial activity on Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi.Both media were prepared according to the manufacturer's instructions, boiled to dissolve, and autoclaved at 121°C for 15 minutes at 15psi.After cooling to 45°C, the sterilized media was aseptically placed into an appropriate number of labeled sterile Petri dishes and allowed to harden.The test organisms were subcultured into Sabouraud Dextrose Broth (SDB) for fungus and Nutrient Broth (NB) for bacteria before being incubated at 37°C for 18-24 hours and 25°C for 24-48 hours, respectively, before being analyzed.

Using the McFarland standard, each organism was standardized to a turbidity of 0.5 x 108 cells/ml in saline solution (0.85% NaCl) (through visual comparison).Using a sterilized cotton swab, 0.1 mm of the standardized suspensions were used to inoculate the surfaces of the 90mm-diameter MHA and SDA plates respectively.Each agar plate was punctured with sterile cork borer tools, each with a 6 mm diameter well. Each hole was filled with a 20mg/ml methanol, ethyl acetate, and n-hexane extract of *F.schweinfurthii*.

Commercial antibiotics (gentamicin and ketoconazole) were employed as positive controls for bacteria and fungi, respectively, to test the sensitivity of the isolates, while DMSO was used as negative controls. After allowing the extract to diffuse into the agar for 5 hours at room temperature, the plates were incubated at 37°C for 18-24h for bacteria and 25°C for 24-48h for fungi, except yeast (*Candida* species), which was incubated at 37°C.The inhibition zones were measured millimeters after incubation using a meter rule. The entire experiment was done three times, and the zone of inhibition's mean values was calculated. 33.

1. **Minimum Inhibitory Concentration Determination**

The MIC was obtained specifically for the extracts and isolates that demonstrated inhibitory action. The MIC of the effective extracts was measured using the broth dilution method33.The extracts were tested against the bacteria at various concentrations ranging from 2500 to 20000 g/ml. The extract-free broth served as a negative control, while the conventional antibacterial drug (gentamycin) served as a positive control. The minimum inhibitory concentration was determined after a 24-hour incubation at 37 °C as the lowest concentration that demonstrated no observable growth using turbidity as a measure.

**Determination of antioxidant activity:**

The free radical scavenging activity of *F. schweinfurthii* methanol, ethyl acetate, and n-hexane extracts was determined *in vitro* using the 2,20-diphenyl-1-picrylhydrazyl (DPPH) test 34.In methanol, sample stock solutions (1.0 mg/mL) were diluted to final concentrations of (500, 250, 125, 62.5g/mL). One mL of a 0.3 mM DPPH ethanol solution was added to 2.5 mL of different concentration sample solutions and left to react at room temperature. After 30 minutes, the absorbance readings at 518 nm were measured and converted to percentage antioxidant activity (AA) using the following formula.DPPH solution (1.0 mL; 0.3 mM) plus methanol (2.5 mL) was used as a control and the reference compound ascorbic acid was also measured. Allmeasurements were made in triplicate and averaged.

 % inhibition = [A control – A sample/A control] x 100.

**Results:**

## Antimicrobial Activity Assay:

Antimicrobial activity of *F. schweinfurthii* aerial parts was tested against gram +ve bacteria (*S. aureus* and *S. epidermidis*), gram -ve bacteria (*E. coli* and *Proteus vulgaris*), and fungal strains (*Trichophyton rubrum* and *Candida albicans*) causing an ear infection. Antimicrobial activity of *F. schweinfurthii* methanol, ethyl acetate, and n-hexane extracts at 20 mg/ml was also compared to conventional antibiotics (Gentamicin) and antifungals (Ketoconazole) and results are provided in Tables1 and 2. All three *F. schweinfurthii* aerial extracts were ineffective against *Trichophyton rubrum* and *Candida albicans*. Positive control showed an inhibitory zone (ketoconazole). The methanol extract was most effective against S. aureus, S. epidermidis, Proteus vulgaris, and E. coli, with inhibition zone values of 15.3±1.21, 9.13±0.75, 13.4±0.8, and 7.8±0.6 mm as compared with other tested extracts. Ethyl acetate extract was less efficient against *S. aureus* (11.2mm), *Proteus vulgaris*(10.3mm), and *E. coli* (7.50mm), With no activity against *S. epidermidis*. In contrast, n-hexane was inactive against all tested microorganisms except *Proteus vulgaris* (11.8± 1.27 mm).

**Table 1**: Antibacterial activity of *F. schweinfurthii*aerial parts extracts at

20 mg/ml.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plant extracts** | ***E. coli*** | ***Proteus vulgaris*** | ***Staphylococcus******epidermidis*** | ***Staphylococcus******aureus*** |
| **Methanol** | 7.8±0.6 | 13.4±0.8 | 9.13±0.75 | 15.3±1.21 |
| **Ethyl acetate** | 7.5±0.5 | 10.3±1.1 | - | 11.2±0.8 |
| **n-hexane** | - | 11.8± 1.27 | - | - |
| **Gentamicin** | 34.6±2.8 | 29.7±2.9 | 33.2±2.4 | 27.5±1.9 |

: Antifungal activity of F. schweinfurthii aerial parts extracts at

20 mg/ml.

|  |  |  |
| --- | --- | --- |
| **Plant extracts** | ***Trichophyton rubrum*** | ***Candida albicans*** |
| **Methanol** | - | - |
| **Ethyl acetate** | - | - |
| **n-hexane** | - | - |
| **Ketoconazole** | 11.8±0.6 | 21.3±1.7 |

**Fig 1.**Antimicrobial activity of *F. schweinfurthii*aerial parts extracts.



A: *Trichophyton rubrum* B: *Candida albicans*



C: *Proteus vulgaris* D: *Escherichia coli*



E: *Staphylococcus aureus* F: *Staphylococcus epidermidis*

**Fig.2:** Inhibition zone of methanol, ethyl acetate and n-hexane extracts of*F.schweinfurthiiaerial parts* against the tested organism.1=Methanol extract , 2= n-hexane extract , 3= Ethyl acetate extract **.**

**Minimum Inhibitory Concentration (MIC):**

The bacterial strains sensitive to *F. schweinfurthii*extracts were tested for the minimum inhibitory concentration (MIC).The MIC for F. schweinfurthii extracts against sensitive bacterial strains is indicated in Table 3 and Fig 3. The minimum inhibitory concentration of methanol extract was 20mg/ml against E. coli and 2.5mg/ml against S. aureus and Proteus vulgaris. In contrast, MIC for S. epidermidis is 10 mg/ml. In comparison, ethyl acetate extract's MIC against *E. coli* was 20 mg/ml, and its lowest MIC against *S. aureus* and *Proteus vulgaris* was 5 mg/ml. On the other hand, n-hexane extract had MICvalue of 5mg/ml against P. vulgaris.

**Table 3:** MIC (mg/ml) of effective F. schweinfurthii extracts against susceptible tested bacterial strains.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Plant extracts | ***E. coli*** | ***Proteus vulgaris*** | ***Staphylococcus******epidermidis*** | ***Staphylococcus******Aureus*** |
| **Methanol** | 20.0 | 2.5 | 10.0 | 2.5 |
| **Ethyl acetate** | 20.0 | 5.0 | - | 5.0 |
| **n-hexane** | - | 5.0 | - | - |
| **Gentamicin** | 0.0024 | 0.0048 | 0.0097 | 0.0048 |

**Fig 3.** Minimum inhibitory concentration of F. schweinfurthii extracts against susceptible microbial strains

**Antioxidant activity:The antioxidant activity of the plant extracts was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant activity of extracts from *F. schweinfurthii* aerial parts was concentration-dependent, with methanol extract having the highest activity level (IC50 = 236 ± 0.2 µg/ml), followed by ethyl acetate extract (IC50 = 351.5± 0.6 µg/ml), and n-hexane extract having no antioxidant activity Table 3 and Fig.4. In contrast to other concentrations examined, the highest DPPH scavenging potential (86.83%, 66.56%) was observed at 500 µg/ml of methanol and ethyl acetate extracts, respectively. In contrast, at 62.5 µg/ml of methanol and ethyl acetate extracts, the lowest DPPH scavenging potential (18.62 ± 0.54%, -9%) was achieved. All the studied extracts had appreciable antioxidant scavenging abilities at lower levels than ascorbic acid.**

**Table 3:**Percentageof DPPH inhibition by *Fagoniaschweinfurthii* Hadidi aerialparts

|  |  |  |  |
| --- | --- | --- | --- |
| Sample Concentration (mg/ml) | Ascorbic acid | Methanol | Ethyl acetate |
| 500 | 95.58±0.03 | 86.84±1.22 a | 66.56±0.21 a |
| 250 | 95.31±0.18 | 59.8±1.02 a | 51.57±0.81 a |
| 125 | 95.14±0.15 | 34.94±3.26 a | 14.37±6.96 a |
| 62.5 | 95.02±0.17 | 18.57±0.25 a | -8.99±7.33 a |

Note: a p value <0.001 compared to reference drug (Ascorbic acid)

**Figure 4.:** DPPH scavenging activity of *F. schweinfurthii extracts*. Ascorbic acid was used as a standard;a p value <0.001 compared to reference drug (Ascorbic acid).

**Table 4** IC50 values of DPPH radical scavenging activity F. schweinfurthiiaerial parts extracts

|  |  |
| --- | --- |
| **Extract** | **IC50 Value (mg/ml)** |
| Methanol  | 236$\pm 0.2$mg/ml |
| Ethyl acetate  | $351.5\pm 0.6 $mg/ml |

**Discussion:**

The systematic and proper screening of numerous extracts derived from various medicinal plants is necessary to search for novel antibiotics.The methanol extract of *F. schweinfurthii* showed potential antibacterial activity against all tested bacterial strains that cause ear infections (*S. aureus, S. epidermidis, Proteus vulgaris,* and *E. coli*). In contrast, the ethyl acetate extract was only effective against *S. aureus, Proteus vulgaris,* and *E. coli*. n-hexane extract, however, was only effective against *Proteus vulgaris*. These results supported those of Shad *et al*29, who found that an n-hexane extract of *F. oliveri*had no effect against *S. aureus, E. coli, P. aeruginosa, S. typhus,* and *B. subtilis*. Similarly, Ur Rehman *et al*.32 reported that methanol extract of *Fagoniacretica* exhibited the greatest antibacterial activity against tested bacterial strains. The methanolic extract demonstrated the highest antibacterial activity, indicating that the polar components of the crude extract predominated over the non-polar ones, confirming the traditional methods of use that rely on the aqueous extract as preferable for public use. However, the findings of this investigation contradicted those of Kouser and Quershi35stated that the methanol extract of *Fagonia indica* has no activity against *S. aureus* or *S. epidermis*. Still, the n-hexane and ethyl acetate extracts show activity against *S. epidermis* with MICs of 2.5 and 1.0 mg/ml, respectively. Similarly, our findings countered those of Shehab *et al*36who demonstrated the antibacterial activity of an n-hexane extract of *Fagonia indica* against *S. aureus* and *E. coli*. Different plant species, growing areas, and bacterial strains could take all account for these antibacterial activity variances.

The presence of tannin, alkaloids, saponins, and flavonoids in *F. schweinfurthii* extracts may explain its antibacterial activity 37, Which is similar to the findings of Doughari& Manzara38 who found a correlation between antibacterial activity and phytoconstituents (alkaloid, saponin, phenol).These phytoconstituents demonstrated antibacterial activity via a different mechanism of action.In immune-compromised individuals, persistent opportunistic fungal infections have become a major cause of morbidity and mortality39. Extracts of *F. schweinfurthii* aerial parts were tested for antifungal activity against *Candida albicans* and *Trichophyton rubrum*. Ketoconazole was employed as an antifungal standard*F. schweinfurthii* aerial parts extracts did not inhibit fungal growth, meaning they are inactive against tested fungal strains. These findings matched those 31,which indicate that ethyl acetate and methanol extracts of *Fagoniamollis* exhibited no antifungal activity against *Aspergillus fumigatus, Aspergillus niger, Candida albicans,* and *Mucor spp*. Similar to Shad *et al*29, methanol extract of *FagoniaOliveri* had no activity *a*gainst *Candida albicans*, and hexane fraction had no activity against many fungal strains tested (*T. longifusus, C. albicans, C. glaberata, F. solani,* and *A. flavus*). These results also agreed with Kouser and Quershi35 who found that n-hexane and methanol extracts do not affect *Candida albicans*.

The current investigation results demonstrated that n-hexane extract lacked antioxidant activity while methanol and ethyl acetate extracts had concentration-dependent antioxidant activity. A similar outcome was obtained by El-Amier& Abo Aisha40 who discovered that as plant extract concentration increased, *F. arabica, F. criticus,* and *F. mollis*methanolic extracts' capacity to scavenge free radicals increased constantly. The maximum antioxidant activity was found in the methanol extract (IC50 = 236 g/ml), followed by the ethyl acetate extract (IC50 = 351.5 g/m), whereas the n-hexane extract had no antioxidant activity.

Our findings on antioxidant activity correspond with those of Pareek *et al*41, which demonstrated that the methanol extract of *F. schweinfurthii* aerial parts had an antioxidant activity with an IC50 value of (200.2 ±7.34µg/ml). The *Fagonialongispina* aerial parts' ethanol extract also demonstrated antioxidant activity, with an IC50 value of (220± 0.0075 µg/ml)28However, the results of the present study contradicted those of a study11, who found that the methanolic extract from *Fagonia indica* aerial parts had weak antioxidant activity (RSA=19± 0.42%). Diverse plant species, locations used for collecting, and extraction techniques could all contribute to this variation in antioxidant activity.Flavonoids and phenolic compounds may be responsible for *F. schweinfurthii's* antioxidant action 42 Additionally, methanol extract of Yemeni *Fagonia indica* leaves has been revealed to include flavonoids and saponins responsible for the antioxidant action 27.

**LIMITATIONS OF THE STUDY**

**Conclusion:**

This study shows that several extracts from *F. schweinfurthii* aerial parts have various antioxidant and antibacterial activities. According to the findings, methanol extract had the highest levels of antibacterial and antioxidant capabilities. Additionally, *F. schweinfurthii* extracts could inhibit bacteria linked to ear infections and may provide scientific support for the plant's traditional usage in folk medicine. Additionally, this plant is expected to be a significant source of natural antioxidants, which might be used as a food complement or to prevent the progression of various oxidative stressors in the pharmaceutical industry.

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**AUTHOR CONTRIBUTIONS:**

All authors agreed to be held accountable for every aspect of the study, helped with data analysis, composed, revised, reviewed the paper, and gave their final approval before publication.

**CONFLICT OF INTEREST**

 "No conflict of interest associated with this work”.

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